# BioScript™ Reverse Transcriptase

Shipping: On Dry/Blue Ice

Catalog numbers

BIO-27036 10,000 units

Batch No.: See vial

BIO-27036-4 4 x 10,000 units

Concentration: 200u/µl

BIOLINE

A Meridian Life Science® Company

**Store at –20°C**

**Storage and stability:**
BioScript Reverse Transcriptase is shipped on dry/blue ice. All kit components should be stored at -20°C upon receipt. Excessive freeze/thawing is not recommended.

**Expiry:**
When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

**Quality control:**
Bioline operates under the ISO 9001 Management System. BioScript Reverse Transcriptase is extensively tested for activity, processivity, efficiency, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

**Safety precautions:**
Please refer to the material safety data sheet for further information.

**Notes:**
Research Use Only.

## Description

BioScript™ is a Moloney Murine Leukaemia Virus (MMLV) Reverse Transcriptase, which exhibits high stability and is active at a wide range of temperatures. Unlike the wild-type enzyme, BioScript possesses low RNase H activity, which results in enhanced yields. In addition, BioScript is highly sensitive even when the amount of template is a limiting factor.

BioScript is suitable for first-strand cDNA synthesis, cDNA library construction, and the production of templates for RT-PCR analysis of gene expression. BioScript can be used with total RNA, mRNA or *in-vitro* transcribed RNA.

## Kit components

| Reagent | 10,000 units | 40,000 units |
|---|---|---|
| BioScript Reverse Transcriptase | 50µl | 4 x 50µl |
| 5x Reaction Buffer | 1.2ml | 4 x 1.2ml |

## Reaction Recommendations and Optimization

**Template Quality**

- Intact, high-quality RNA is essential for the reverse-transcription reaction.
- All reagents for use with RNA must be prepared using DEPC-treated water (BIO-38030).
- The inclusion of an RNase Inhibitor can reduce template degradation and increase yield of PCR product (BIO-65027).
- Low-copy-number genes may require an increase in starting material.
- It is necessary to use a suitable RNA extraction reagent e.g., TRIsure™ (BIO-38032) or ISOLATE II RNA Isolation Kit (BIO-52072).

**Primer Design and Concentration**

There are three methods for priming cDNA synthesis:

- **Oligo dT Primers**
  Oligo dT priming (BIO-38029) uses the poly-A tail found on the 3' end of most eukaryotic mRNAs. This ensures that the 3' end of mRNAs are represented, although long mRNAs can have their 5' ends under-represented in the subsequent cDNA pool. Use at 0.5µM final concentration.

- **Random Hexamers**
  Random priming (BIO-38028) gives random coverage to all regions of the RNA to generate a cDNA pool containing various lengths of cDNA. Random priming is unable to distinguish between mRNA and other RNA species present in the reaction. Use at 2.0µM final concentration.

- **Gene Specific Primers (GSP)**
  Gene specific primers are designed to generate cDNA for a specific gene of interest. It is a widely used method for performing One-Step RT-PCR when only 1 gene is under investigation. It can be useful when RNA concentrations are low. Use at 0.4µM final concentration.

A combination of Oligo dT and Random Hexamers primers can improve the reverse transcription efficiency of some mRNA templates.

**Extension Temperature**

- Efficient reverse-transcription can be achieved at temperatures of 37°C to 42°C for 30-60 min.
- The use of higher incubation temperatures up to 45°C may increase the yield of cDNA synthesized in cases of complex RNA secondary structure. However, the yield of the majority of RNA molecules will be reduced.

## BioScript Reverse Transcription Protocol

1. Vortex solutions and centrifuge briefly before use.
2. Prepare the priming premix on ice in an RNase-free reaction tube:

| | |
|---|---|
| Total RNA (up to 5µg) or mRNA (up to 0.5µg) | *n*µl |
| Primer: Oligo $(dT)_{18}$ (10µM) *or* Random Hexamer (40µM) *or* GSP (8µM) | 1µl |
| 10mM dNTP mix | 1µl |
| DEPC-treated Water | up to 10µl |

3. Incubate samples at 70°C for 5 min, then chill on ice for at least 1 min.
4. Prepare the reaction premix:

| | |
|---|---|
| 5x RT Buffer | 4µl |
| RiboSafe RNase Inhibitor | 1µl |
| BioScript Reverse Transcriptase (200u/µl) | 1µl |
| DEPC-treated Water | to 10µl |

5. Add 10µl of the reaction premix to the priming premix and mix gently by pipetting.
6. Incubate samples at 42°C for 30 min. If using random hexamers, incubate 10 min at 25°C followed by 42°C for 30 min.
7. Terminate reaction by incubating at 85°C for 5 min, chill on ice.
8. Store reaction at –20°C for long term storage, or proceed to PCR immediately.

**This protocol is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimization.**

## Troubleshooting

| Problem | Possible Cause | Recommendation |
|---|---|---|
| No cDNA synthesis | RNA degraded | Analyze RNA on a denaturing gel to verify integrity. Ensure that all reagents are RNase-free. Use RiboSafe RNase inhibitor in the first-strand reaction (BIO-65027). |
| | RNA contained an RT inhibitor | The presence of inhibitors can be determined by mixing a control RNA with some of the sample and comparing the yield with that of the original amplification. Remove inhibitors such as SDS, EDTA, formamide and pyrophosphate, by ethanol precipitation of RNA, including a 70% ethanol wash step. |
| | Reaction temperature not optimal | Perform a temperature-gradient experiment ranging from 37-45°C. |
| | Not enough starting RNA | Increase the amount of starting RNA, this can be an important factor when amplifying low-copy genes from total RNA. |
| | RNA had high secondary structure | Prior to reaction set-up, denature RNA with primers. Raise the temperature of the RT step, up to a maximum of 45°C (for short amplicons). |
| | Insufficient product | Increase reverse transcription step to 60 minutes |
| Poor Specificity in PCR | Non-specific annealing of primers to template | Use gene-specific primers rather than Oligo dT or random hexamers in RT reaction.<br>Increase the annealing temperature in PCR.<br>Check for presence of pseudogenes.<br>Set up reactions on ice. |
| | Primer dimers | Redesign primers to prevent self-annealing. |
| | Genomic DNA contamination | Treat RNA with DNase I and re-purify. If possible, use intron-spanning primers in PCR. |
| Product in no-RTase control | Template contaminated with DNA | Treat samples with DNase I. |

## Technical Support

If the troubleshooting guide does not solve the difficulty you are experiencing, please contact Technical Support with details of reaction setup, cycling conditions and relevant data.

Email: tech@bioline.com

## Associated products:

| Product Name | Cat. No. |
|---|---|
| RiboSafe RNase Inhibitor | BIO-65027 |
| TRIsure™ | BIO-38032 |
| ISOLATE II RNA Mini Kit | BIO-52072 |
| Random Hexamer Primer | BIO-38028 |
| Oligo (dT)18 Primer | BIO-38029 |
| dNTP Mix (10mM) | BIO-39053 |
| SensiFAST™ cDNA Synthesis Kit | BIO-65053 |
| SensiFAST™ SYBR No-ROX Kit | BIO-98005 |
| Agarose, Molecular Grade | BIO-41026 |

## Product Citations


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5. Bertram, S. *et al. J. Virol.* **84**, 10016-10025 (2010).
6. Shemesh, Y. *et al. J. Neurosci.* **30**, 12517-12525 (2010).
7. Cottage, A. *et al. J. Exp. Bot.* **61**, 3773-3786 (2010).
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